Type and amount of dietary protein in the treatment of metabolic syndrome: a randomized controlled trial[1,2]

*Alison M Hill,[3,4] Kristina A Harris Jackson,[4] Michael A Roussell,[4] Sheila G West,[4,5] and Penny M Kris-Etherton[4]**

[3]School of Pharmacy & Medical Sciences, University of South Australia, Adelaide, Australia; and [4]Department of Nutritional Sciences and [5]Department of Biobehavioral Health, The Pennsylvania State University, University Park, PA

ABSTRACT

Background: Food-based dietary patterns emphasizing plant protein that were evaluated in the Dietary Approaches to Stop Hypertension (DASH) and OmniHeart trials are recommended for the treatment of metabolic syndrome (MetS). However, the contribution of plant protein to total protein in these diets is proportionally less than that of animal protein.

Objective: This study compared 3 diets varying in type (animal compared with plant) and amount of protein on MetS criteria.

Design: Sixty-two overweight adults with MetS consumed a healthy American diet for 2 wk before being randomly allocated to either a modified DASH diet rich in plant protein (18% protein, two-thirds plant sources, $n = 9$ males, 12 females), a modified DASH diet rich in animal protein (Beef in an Optimal Lean Diet: 18.4% protein, two-thirds animal sources, $n = 9$ males, 11 females), or a moderate-protein diet (Beef in an Optimal Lean Diet Plus Protein: 27% protein, two-thirds animal sources, $n = 10$ males, 11 females). Diets were compared across 3 phases of energy balance: 5 wk of controlled (all foods provided) weight maintenance (WM), 6 wk of controlled weight loss (minimum 500-kcal/d deficit) including exercise (WL), and 12 wk of prescribed, free-living weight loss (FL). The primary endpoint was change in MetS criteria.

Results: All groups achieved ~5% weight loss at the end of the WL phase and maintained it through FL, with no between-diet differences (WM compared with WL, FL, $P < 0.0001$; between diets, P = NS). All MetS criteria decreased independent of diet composition (main effect of phase, $P < 0.01$; between diets, P = NS). After WM, all groups had a MetS prevalence of 80–90% [healthy American diet (HAD) compared with WM, P = NS], which decreased to 50–60% after WL and was maintained through FL (HAD, WM vs WL, FL, $P < 0.01$).

Conclusions: Weight loss was the primary modifier of MetS resolution in our study population regardless of protein source or amount. Our findings demonstrate that heart-healthy weight-loss dietary patterns that emphasize either animal or plant protein improve MetS criteria similarly. This study was registered at clinicaltrials.gov as NCT00937638. *Am J Clin Nutr* 2015;102:757–70.

Keywords: dietary protein, metabolic syndrome, lean beef, weight loss, body composition

INTRODUCTION

Metabolic syndrome (MetS)[6] is characterized by a clustering of cardiovascular disease (CVD) risk factors, and as the number and severity of these increase, so does the risk of CVD, type II diabetes, and all-cause mortality (1, 2). Treatment of MetS includes weight loss to reduce abdominal obesity, a healthy dietary pattern, and regular physical activity (3). A weight loss of 5–10% is associated with substantial improvements in blood glucose, triglycerides, and blood pressure (BP), as well as LDL cholesterol and HDL cholesterol (4). Because weight loss and especially maintenance of weight loss are challenging for many individuals, a dietary pattern that improves MetS criteria independent of weight loss could beneficially affect risk of MetS comorbidities. A Dietary Approaches to Stop Hypertension (DASH) dietary pattern is recommended for LDL cholesterol and BP lowering (5). Variations in the macronutrient profile of the DASH diet that emanate from the OmniHeart trial are recommended for the treatment of MetS criteria (6).

The DASH dietary pattern decreased BP (7) and LDL cholesterol (8, 9) compared with a control diet (which was lower in total protein and higher in total fat and SFAs). The OmniHeart trial demonstrated that diets low in SFAs and higher in unsaturated fat or protein improved BP and beneficially affected HDL cholesterol and triglycerides, which typically are adversely affected by a lower fat/higher carbohydrate diet (6). Both the DASH (7–9) and OmniHeart

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[2] Supplemental Material is available from the "Supplemental data" link in the online posting of the article and from the same link in the online table of contents at http://ajcn.nutrition.org.

*To whom correspondence should be addressed. E-mail: pmk3@psu.edu.

[6] Abbreviations used: BOLD, Beef in an Optimal Lean Diet; BOLD+, Beef in an Optimal Lean Diet Plus Protein; BP, blood pressure; CVD, cardiovascular disease; DASH, Dietary Approaches to Stop Hypertension; DBP, diastolic blood pressure; DXA, dual-energy X-ray absorptiometry; FL, free-living weight-loss phase; HAD, healthy American diet; HR, heart rate; M-DASH, modified Dietary Approaches to Stop Hypertension; MetS, metabolic syndrome; RHI, reactive hyperemia index; SBP, systolic blood pressure; TC, total cholesterol; WC, waist circumference; WL, weight loss (minimum 500-kcal/d deficit) including exercise phase; WM, weight-maintenance phase.

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trials (6) support cardiovascular benefits of a plant-based diet, including an emphasis on plant protein (5). Of note, the DASH dietary pattern includes substantial quantities of animal protein from reduced-fat dairy products, seafood, and white meats, and although increased, the contribution of plant protein to overall protein is proportionally less (10). Moreover, the moderate-protein diet used in the OmniHeart trial consisted of >50% animal protein (6, 11). We have shown recently that when SFAs remain low (<7% of total calories), average and moderate-protein diets containing lean beef [Beef in an Optimal Lean Diet (BOLD)] also can be included in a heart-healthy dietary pattern that lowers LDL cholesterol and BP (12, 13).

The purpose of this study was to compare 3 diets controlled for SFAs with varying amounts of protein from plant and animal (predominantly lean beef) sources on MetS criteria (primary endpoint): a modified-DASH (M-DASH) diet rich in plant protein (18% protein, two-thirds plant sources), an M-DASH diet rich in animal protein (BOLD: 18.4% protein, two-thirds animal sources), and a moderate-protein diet [Beef in an Optimal Lean Diet Plus Protein (BOLD+): 27% protein, two-thirds animal sources]. These diets were compared at 3 phases of energy balance: controlled weight maintenance (WM), controlled weight loss with an exercise component (WL), and prescribed free-living weight loss (FL). Secondary outcomes were endothelial function, LDL cholesterol, and adiposity.

METHODS

Participants

Overweight and obese [BMI (in kg/m^2): 27–42] men and women 30–60 y of age with MetS were recruited. MetS was defined according to National Cholesterol Education Program Adult Treatment Panel III criteria (14) with participants having ≥3 of the following criteria: abdominal obesity [waist circumference (WC) >102 cm (40 inches) in men and >88 cm (35 inches) in women], elevated blood glucose [>100 mg/dL (5.6 mmol/L)], elevated triglycerides [>150 mg/dL (1.7 mmol/L)], low HDL cholesterol [<40 mg/dL (1.03 mmol/L) in men and <50 mg/dL (1.29 mmol/L) in women], and hypertension [systolic blood pressure (SBP) >130 mm Hg and/or diastolic blood pressure (DBP) >85 mm Hg]. Pharmacologic treatment of any of these criteria, except for abdominal obesity, was considered a MetS criterion.

Participants taking a single oral BP-lowering drug were eligible for the study as long as their screening BP was <160/100 mm Hg. BP medication was allowed throughout the study. Participants taking a single cholesterol or glucose medication were eligible for screening, but, in consultation with their primary care physician, they discontinued use of these medications before beginning the study.

All participants were nonsmokers and free of established CVD, stroke, diabetes, or liver, kidney, or autoimmune disease. Exclusion criteria included continued use of glucose and cholesterol/lipid-lowering medication or supplements (psyllium, fish oil, soy lecithin, and phytoestrogens), pregnancy or lactation, weight loss of ≥10% of body weight within the 6 mo before enrolling in the study, high alcohol consumption (≥14 drinks/wk), participation in regular physical activity (>1 formal session/wk) with the intention of losing weight or increasing fitness, inability to complete the exercise testing protocol as determined by the clinic physician, orthopedic or other health issues that precluded treadmill exercise or involvement in the pedometer-based walking program, vegetarianism, and lactose intolerance. All participants were informed that 2 of the diets contained lean beef.

The institutional review board at The Pennsylvania State University approved the experimental protocol, and all participants provided written informed consent. This study was registered at clinicaltrials.gov as NCT00937638.

Study design

This was a 6-mo, randomized, parallel-arm, open-label, controlled-feeding trial comparing the effects of different sources and amounts of dietary protein on MetS prevalence. All participants completed a 2-wk controlled-feeding (all food and drinks were prepared by a Metabolic Kitchen and provided to participants) healthy American diet (HAD) run-in where weight was held stable. Energy requirements for this phase were initially estimated with the Harris-Benedict equation (15) multiplied by an activity factor of 1.5 for men and 1.3 for women and modified as required based on changes in body weight as determined by daily weigh-ins at the Metabolic Diet Study Center. The end of the HAD phase was considered the baseline. Participants were then blocked in groups of 3 by BMI, sex, and age and randomly allocated by computer-generated assignment to one of 3 experimental treatments (diets): M-DASH, BOLD, and BOLD+. These diets were compared at different levels of energy balance. Participants first consumed one experimental diet for 5 wk at energy equilibrium (controlled-feeding weight maintenance; WM). If desired, a short compliance break (1 wk) was taken before completing a 6-wk controlled-feeding weight-loss phase (WL) where an energy deficit was induced by calorie reduction (minimum 500-kcal/d deficit through dietary changes) and increased physical activity via a walking program. Participants consumed the same experimental diets during the WM and WL phases. Participants then completed a 12-wk free-living weight-loss phase (FL), during which time they were asked to continue their assigned hypocaloric diets and physical activity, but the provision of food and drinks was discontinued. Three 90-min one-on-one nutrition education sessions with a registered dietitian were conducted for all participants during the controlled-feeding weight-loss phase in preparation for the free-living phase. Participants were educated on the unique features of their assigned diets—namely, the incorporation of increased vegetable or animal protein from specific food sources. They also were educated about the principles of healthy eating, recommended portion sizes for food groups (using food models), and provided with practical guidelines to assist them in selecting foods that were appropriate for their diet. Strategies to achieve their target calorie amount—for example, the contribution of discretionary foods and beverages (including alcohol) to energy intake—also were discussed. To assist participants with adhering to both their calorie amount and their experimental diet, they were provided with the menus and recipes used by the Metabolic Kitchen during their final week of controlled weight loss.

Participants completed a series of clinical and physical assessments on 2 consecutive days at baseline (end of HAD) and at the end of the WM, WL, and FL phases. At each visit, participants arrived in the fasting state (12 h water only, 48 h no alcohol, and 12 h without vigorous exercise) at the Clinical Research Center where body weight, WC, vascular function (by EndoPAT; Itamar Medical), BP, and blood samples (~30 mL on each day) were

obtained. Height was measured at baseline (after HAD). Body composition was measured by dual-energy X-ray absorptiometry (DXA) at baseline (end of HAD) and at the end of the WL and FL phases. Two single-day visits were completed during weeks 4 and 8 of the 12-wk FL phase. The purpose of these visits was to monitor weight (not included in the analyses) and address any concerns that subjects had about their diet and physical activity.

Participants could not be blinded to their dietary assignment. An unblinded study coordinator blocked participants and conducted all data analyses. Outcome assessors (i.e., nurses and technicians) were blinded.

Diets

The nutrient composition of the experimental diets is shown in **Table 1**. The 3 experimental diets (M-DASH, BOLD, and BOLD+) were matched for total fat, SFAs, MUFAs, PUFAs, cholesterol (<300 mg/d), sodium, potassium, calcium, and magnesium. The M-DASH diet was higher in fiber (55 compared with 38 g), a consequence of incorporating more plant protein from whole-food sources such as pulses (beans) and whole grains, which also were rich in fiber. The HAD was higher in total fat, SFAs, cholesterol, and sodium and was lower in total fiber, potassium, calcium, and magnesium. The BOLD and M-DASH diets were matched for macronutrient composition but differed in the relative contribution of plant and animal protein to total protein. Animal protein (from lean beef, chicken, tuna, eggs, and dairy) contributed two-thirds of the total protein on the BOLD diet, whereas two-thirds of the total protein was from plant sources (pulses, grains, soy, nuts, and seeds were substituted for lean beef protein) on the M-DASH diet. To isolate the effect of removing red meat and SFAs rather than the substitution of functional, cholesterol-lowering foods on LDL cholesterol, we limited soy protein to <10% of total protein, and psyllium and margarines containing sterols/stanols were not included. The BOLD+ diet was higher in protein (27% of total energy) compared with HAD (17.4%), M-DASH (18%), and BOLD (18.4%) diets and thus lower in carbohydrate (45% compared with 49–55%). Animal protein contributed two-thirds of the total protein on the BOLD+ diet.

TABLE 1
Nutritional composition of experimental diets[1]

	HAD	M-DASH	BOLD	BOLD+
Energy, kcal	2104	2100	2097	2105
Protein	17.4 (91.4)[2]	17.9 (99.7)	18.4 (102.6)	27 (149.2)
Plant source	42.0 (38.4)	64.5 (64.3)	37.9 (38.9)	31.6 (47.1)
Animal source	58.0 (53.1)	35.5 (35.4)	62.1 (63.7)	68.4 (102.2)
Carbohydrate	49.4 (260.0)	55 (288.8)	54 (283.0)	45 (236.8)
Fat	33.2 (77.6)	27 (63)	27 (62.9)	27 (63)
Cholesterol, mg	233	89	152	195
SFAs	13.3 (31.1)	6.6 (15.3)	6.1 (14.3)	6.1 (14.3)
PUFAs	5.4 (12.7)	5.8 (13.5)	6.3 (14.7)	6.3 (14.7)
MUFAs	11.3 (26.3)	11.5 (26.9)	10.7 (24.8)	11.6 (27)
Fiber, g	21	55	38	38
Sodium, mg	3640	2700	2666	2789
Potassium, mg	2380	4112	4241	4328
Calcium, mg	450	1282	1436	1380
Magnesium, mg	227	462	464	459
Lean beef, g (oz/d)	39.7 (1.4)[3]	11.7 (0.4)	139 (4.9)	196.2 (6.9)

[1]Based on 2100 kcal/d, averaged across a 6-d menu cycle. All values were determined with NUTRTIONST PRO (Axxya Systems LLC). BOLD, Beef in an Optimal Lean Diet; BOLD+, Beef in an Optimal Lean Diet Plus Protein; HAD, healthy American diet; M-DASH, modified Dietary Approaches to Stop Hypertension Diet.

[2]Values for macronutrients are percentage of calories, except for protein sources, which are percentage of total protein; values in parentheses are grams (all such values).

[3]Values are grams; ounces in parentheses.

The HAD contained full-fat cheese and dairy products, more vegetable oil and butter, and refined grains. The M-DASH, BOLD, and BOLD+ diets contained low-fat or nonfat versions of these foods, less oil and butter, and more whole grains. All diets were rich in fruits, vegetables, and lean meats, consistent with food-based dietary recommendations. The BOLD and BOLD+ diets included a serving of lean beef each day. More specifically, of the 14 lunch/dinner meals provided for a week, 11 meals included lean beef, 2 contained chicken, and 1 contained fish. However, the BOLD+ diet contained more lean beef than did the BOLD diet (196 compared with 139 g/d). The amount of lean beef in the BOLD diet is consistent with the recommendations from the Third Adult Treatment Panel for the Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults for lean beef consumption for the Step 2 diet (16). In comparison, the M-DASH included 1 lean beef–based, 3 chicken-based, 1–2 fish-based, 7 pulse/vegetable-based, and 1–2 soy-based meals per week. The HAD and M-DASH diet contained 40 and 12 g lean beef/d, respectively. The contribution of protein from various whole-food sources for each of the experimental diets (M-DASH, BOLD, and BOLD+) is shown in **Table 2**. Protein from lean beef provided ~7%, 62%, and 56% of total animal protein for the M-DASH, BOLD, and BOLD+ diets, respectively. The BOLD+ diet provided more lean beef as well as more protein, and therefore lean beef contributed proportionally less to animal protein than the BOLD diet.

The lean beef used in the study was purchased from The Pennsylvania State University Meats Laboratory and primarily included select grade top round, ribeye, chuck shoulder pot roast, and 95% lean ground beef. The meat was prepared via braising, grilling, or frying (95% lean ground beef only) and never over an open flame to prevent charring.

A 2100-kcal menu served as the basis for the other menus that provided a range of calorie amounts (1800–3900 kcal/d in 300-kcal increments); all foods were increased or decreased proportionally such that no single food was removed, and the foods provided were similar across all calorie amounts. A 1600-kcal menu was developed for some female participants during the WL phase. Menus were created for a 6-d cycle that was repeated throughout the controlled-feeding phases. The 6-d rather than the 7-d menu cycle ensured that the same menu was not consumed on the same day of the week throughout the controlled-feeding phases (e.g., every Monday was pot roast for dinner for participants in the BOLD+ group). To induce a calorie deficit during the controlled WL phase, we gave individuals a lower calorie menu. The dietary changes were designed to induce a minimum 500-kcal/d deficit. Sample daily menus for each of the diets are provided in **Table 3**. All meals and snacks were prepared for the controlled-feeding phases of the study at the Metabolic Diet Study Center at The Pennsylvania State University. Participants ate one meal per day (Monday–Friday) in the diet center, and their other meals were prepared and packed for off-site consumption for the remaining weekday and weekend meals. Participants were allowed one "free meal" on holidays

TABLE 2
Dietary protein sources in the experimental diets[1]

	M-DASH	BOLD	BOLD+
Total protein, g	99.7	102.6	149.2
Animal source	35.4 (35.5)[2]	63.7 (62.1)	102.2 (68.4)
Lean beef protein	2.5 (7.2)	39.6 (62.2)	57.1 (55.8)
Other meat protein	12.6 (35.5)	8.3 (13.1)	19.7 (19.3)
Dairy protein	20.3 (57.3)	15.7 (24.7)	25.4 (24.9)
Plant source	64.3 (64.5)	38.9 (37.9)	47.1 (31.6)
Soy protein	7.1 (11.0)	0.0 (0)	6.5 (13.8)
Pulses	8.1 (12.6)	3.3 (8.5)	3.7 (7.8)
Nuts and seeds	11.2 (17.4)	6.3 (16.3)	9.5 (20.2)
Fruits and vegetables	11.0 (17.1)	12.8 (33.0)	10.4 (22.2)
Grains	26.9 (41.8)	16.4 (42.3)	17.0 (36.1)

[1]Based on 2100 kcal/d, averaged across a 6-d menu cycle. All values were determined with NUTRTIONST PRO (Axxya Systems LLC). BOLD, Beef in an Optimal Lean Diet; BOLD+, Beef in an Optimal Lean Diet Plus Protein; HAD, healthy American diet; M-DASH, modified Dietary Approaches to Stop Hypertension Diet.

[2]Grams; percentage of total animal or plant source in parentheses (all such values).

(e.g., July 4th); for these meals, they were given guidance about sensible eating, including avoiding overconsumption and excessive alcohol intake.

Compliance with the experimental diets during the controlled-feeding phases was monitored via daily questionnaires (**Supplemental Material**) asking about the consumption of study and nonstudy foods and beverages, as well as weigh-ins. Participants were classified as noncompliant on any day when they consumed a nonstudy food or beverage or did not consume a study food or beverage. Compliance to each controlled-feeding phase was determined by dividing the number of noncompliant days by the number of days reported for each diet group; this excluded the free meals allowed (e.g., holiday meals). This method did not capture the degree to which participants were noncompliant because any deviation was simply tabulated as a noncompliant day; for example, a participant who replaced the entire study meal with pizza and breadsticks and a participant who did not consume the tahini dressing on a study salad at lunch were both classified as noncompliant on that day. For this reason, we considered changes in body weight as an additional measure of compliance.

The term *adherence* is used to describe how well participants followed the advice to continue to lose weight via dietary changes and exercise during the FL phase. This term implies that participants were in agreement with the treatment regimen and goals, but unlike the controlled-feeding phases, there was some flexibility in how participants implemented strategies to achieve their goals. To reproduce the daily monitoring implemented during the controlled-feeding phases would have placed substantial burden on participants during this phase of the study. Therefore, we based our interpretation of adherence during the FL phase on changes in body weight and MetS criteria.

Physical activity

All participants underwent a 12-lead electrocardiogram-controlled graded submaximal walking test as part of the prescreening for entry to the study. Treadmill speed and gradient were modified each minute until participants reached 85% of their age-predicted maximum heart rate (HR) (220 beats/min − age). During the walking test, participants were under permanent electrocardiogram monitoring, and BP was taken at rest and every 3 min. Oxygen uptake at 85% maximal HR was determined via the following

TABLE 3
Example of 1-d menus for the test diets[1]

	HAD	M-DASH	BOLD	BOLD+
Breakfast	Pancakes with butter and light syrup Peaches, canned in juice Cottage cheese (1%) Apple juice	Pancakes with butter and light syrup Blueberries Skim milk Orange juice	Bran flakes with raisins and skim milk Whole-wheat mini-bagel and margarine Orange juice Banana	Bran flakes with raisins and skim milk Cottage cheese (1%) Orange juice
Lunch	Turkey, provolone cheese, and lettuce sandwich on white bread with mayonnaise Granola bar	Spinach/baby greens salad with cherry tomatoes, mandarin oranges, grilled chicken breast, and dressing Edamame beans Whole-wheat dinner roll with butter Pistachios	Barbeque beef sandwich on whole-wheat bun Spinach salad with cherry tomatoes and dressing Thin pretzels Pear	Beef chili with shredded cheddar cheese (low fat) and whole-wheat crackers Peaches, canned in juice
Dinner	Szechuan stir-fry entrée with pork and white rice White dinner roll with butter Romaine lettuce salad with carrots and Italian dressing	Ratatouille (eggplant/peppers) with pasta Spinach salad with carrots, cherry tomatoes, red bell pepper, chickpeas, and dressing	Spinach and beef skillet with ribeye steak Brown rice Mixed baby greens salad with carrots, cherry tomatoes, and dressing	Pot roast with mashed potatoes and gravy White dinner roll with margarine Broccoli and edamame beans Romaine salad with cherry tomatoes and dressing
Snack	Plain bagel with cream cheese	Light yogurt High-fiber cereal Almonds	Light yogurt Orange Almonds	Hummus with whole wheat pita and baby carrots Trail mix

[1]BOLD, Beef in an Optimal Lean Diet; BOLD+, Beef in an Optimal Lean Diet Plus Protein; HAD, healthy American diet; M-DASH, modified Dietary Approaches to Stop Hypertension.

calculation: $\dot{V}O_2$ (mL/kg per minute) = (0.1 × S) + (1.8 × S × G) + 3.5 mL/kg per minute (where S = speed in m/min and G = percent grade expressed as a fraction) (17). At the end of the WL and FL phases, participants underwent the same fitness assessment to evaluate physiologic changes induced by exercise.

Participants were provided with a pedometer (SW-700 Digiwalker; New Lifestyles) and a personalized pedometer-based walking program that was intended to increase their amount of physical activity to 10,000 steps/d or more by the end of the WL phase. To establish their baseline, participants recorded their daily steps during the last 2 wk of the controlled-feeding WM phase. These step counts were used to guide the development of a personalized walking program, which increased progressively in volume (steps/d) and intensity (steps performed at moderate-vigorous intensity) throughout the 6-wk WL phase. Participants recorded their daily steps throughout the WL phase (energy expenditure via physical activity was not determined). Participants were advised to maintain 10,000 steps/d during the FL phase and, if desired, to incorporate additional physical activities.

Clinical assessments

Body weight was measured at each clinical visit at the Clinical Research Center (in addition to daily weigh-ins at the diet center). WC was measured according to the *NHANES Anthropometry Procedures Manual* as defined by the CDC (18). Body composition was determined by DXA: participants weighing <157 kg were measured with Hologic DXA (QDR-4500W; Hologic Corporation), and those weighing >157 kg were measured with a GE Lunar iDXA (General Electric). Participants were scanned while wearing cotton shorts and T-shirt and while in the supine position in accordance with the manufacturer's instructions. Abdominal fat was calculated by inserting a 50-cm^2 region of interest around the center point of the midline between the lateral iliac crests and the lowest rib margins. The abdominal region of interest could not be measured with iDXA; therefore, abdominal fat was not assessed for one individual from each of the diets (i.e., M-DASH, BOLD, and BOLD+, $n = 3$). Three participants with incomplete data also were not analyzed. The DXA scanners were calibrated according to the standard procedures recommended by the manufacturer. BP (3 repeat measurements spaced 1 min apart) was measured with participants in a seated position after a minimum 5-min rest period, at baseline, and at the end of each phase. Endothelial function [reactive hyperemia index (RHI), Framingham RHI] and vascular stiffness (augmentation index, augmentation index normalized to an HR of 75 beats/min) were measured with pulse amplitude tonometry (Itamar Medical) as previously described (12). HR was measured by the pulse amplitude tonometry device as beats per minute.

Biochemical assessments

Serum and plasma aliquots from fasting blood samples were stored at −80°C until time of analysis. For the first 19 enrolled participants, samples were shipped frozen and analyzed in the core endocrine laboratory at the Milton S. Hershey Medical Center (Hershey, PA). Total cholesterol (TC) and triglycerides were measured by using enzymatic procedures with commercially available kits (Alfa Wassermann). HDL cholesterol was quantified according to the modified heparin-manganese precipitation procedure of Warnick and Albers (19). LDL cholesterol was calculated with the Friedewald equation: LDL cholesterol = TC – HDL cholesterol – (triglycerides ÷ 5) (20). Glucose was determined by an immobilized enzyme biosensor for glucose with the YSI 2300 STAT Plus Glucose & Lactate Analyzer (Yellow Springs Instruments). For the subsequent participants, lipids and glucose were measured in fresh samples by Quest Diagnostics by enzymatic procedures and spectrophotometry. The CVs for TC, HDL cholesterol, and triglycerides were <2%. For all participants, insulin was quantified by radioimmunoassay (Quest Diagnostics). Serum C-reactive protein was measured by latex-enhanced immunonephelometry (Quest Diagnostics; assay CV <8%).

Statistical analysis

All statistical analyses were performed with SAS (version 9.2; SAS Institute). Screening values (means ± SDs) for the treatment groups were compared with a nonparametric 2-sided *t* test (PROC NPAR1WAY). Differences between treatment groups after the HAD run-in diet (means ± SEMs) were assessed with linear-mixed models (PROC MIXED). Normality of the variables at each time point was assessed, and variables were log-transformed if skewed. Means ± SEMs are presented for normally distributed variables; nonnormally distributed variables are presented as medians and 95% CIs. Repeated-measure ANCOVA (repeated for phase) was used to test the effects of treatment (diet) and phase (WM, WL, and FL) on the outcome variables, adjusting for age and sex. A doubly repeated-measure ANCOVA (repeated for phase and day of blood draw) was used to determine the effects of treatment and phase on lipids and lipoproteins, adjusting for age and sex. The model fit was determined by selecting the best covariance structure (compound symmetry, autoregressive 1, and unstructured) for each endpoint as determined by the lowest Bayesian information criterion and the normality of the model residuals. Interaction and main effects were considered statistically significant at $P < 0.05$ and trends at $P < 0.1$. Tukey-Kramer adjusted *P* values were used to determine where the post hoc differences occurred within statistically significant interaction or main effects, with significance set at $P < 0.05$. Multiple models comparing group differences were analyzed (raw values at all time points, raw values adjusted for baseline, and change scores), and all provided similar results. Adjustment of *P* values for multiple outcome variables by using the Benjamini-Hochberg-Yekutieli procedure (21) did not change results; therefore, unadjusted *P* values are presented. Multivariate logistic regression analysis was used to evaluate the relation between the independent variables diet, weight loss (percent change from HAD or WM to end WL or end FL), age, and sex and the dependent variable resolution of MetS. In this analysis, presence of MetS was coded as 0, and resolution of MetS was coded as 1.

Power calculations were based on studies comparing the effects of different amounts or sources of dietary protein on BP in hypertensive populations (6, 7, 22) and triglycerides in hypercholesterolemic populations (23). The estimated effect sizes for the M-DASH and BOLD diets (7, 22) were reductions of 5.5–10.7 mm Hg SBP and 3.0–4.7 mm Hg DBP. The estimated effect size for the BOLD+ diet (6) was a reduction of 9.5 mm Hg SBP and 5.2 mm Hg DBP. The effect size of the higher animal protein diets (BOLD, BOLD+) for triglycerides was calculated to be a reduction of 19–25% (23). Because the experimental diets had not been compared before in the literature, we could not estimate

between-group differences. For 80% power to detect statistically significant within-group changes from HAD (baseline) to WM for BP and triglycerides at an α level of 0.05, a final sample size of 84 participants (28 per group) was needed. The recruitment goal was 90 participants (30 per group) to account for dropouts. Cohen's *d* effect sizes were calculated for BP data (change scores, WM-HAD) by using the procedures outlined by Thalheimer and Cook (24).

RESULTS

Recruitment of study participants took 2.5 y because of the strict study eligibility criteria. Of the 572 respondents, 180 (31%) completed the informed consent and were screened at the clinic for eligibility. Between November 2008 and March 2011, 73 (13%) were enrolled and began the HAD diet (**Figure 1**). Seven participants did not complete the HAD run-in diet. The remaining 66 individuals were randomly allocated, after which 7 participants did not complete the study because of an inability to comply with the diet ($n = 3$), unrelated illness ($n = 2$), relocation ($n = 1$), and scheduling conflicts ($n = 1$). Of these 7 participants, 3 completed an entire feeding phase before dropping out, and their data are included in the final analysis ($n = 62$). The retention rates for the 3 diet groups were similar: 95% ($n = 20/21$) for BOLD, 95% ($n = 21/22$) for BOLD+, and 91% ($n = 21/23$) for M-DASH. There were no differences among groups at screening (**Table 4**). One participant (M-DASH) was taken off BP medications before starting the study and maintained acceptable BP to remain in the study; 7 participants (BOLD, $n = 2$; BOLD+, $n = 2$; M-DASH, $n = 3$) were taking BP medications during the study. Because of difficulties in recruitment for a 6-mo dietary intervention study, recruitment goals were not met.

Dietary and exercise compliance

Daily compliance records for the controlled-feeding HAD phase indicated that there was total dietary compliance on 74% ± 2%, 81% ± 3%, and 84% ± 1% of reported study days for the BOLD, BOLD+ and M-DASH groups, respectively. During the WM phase (when participants were consuming the experimental diets), participants in the BOLD, BOLD+, and M-DASH groups reported

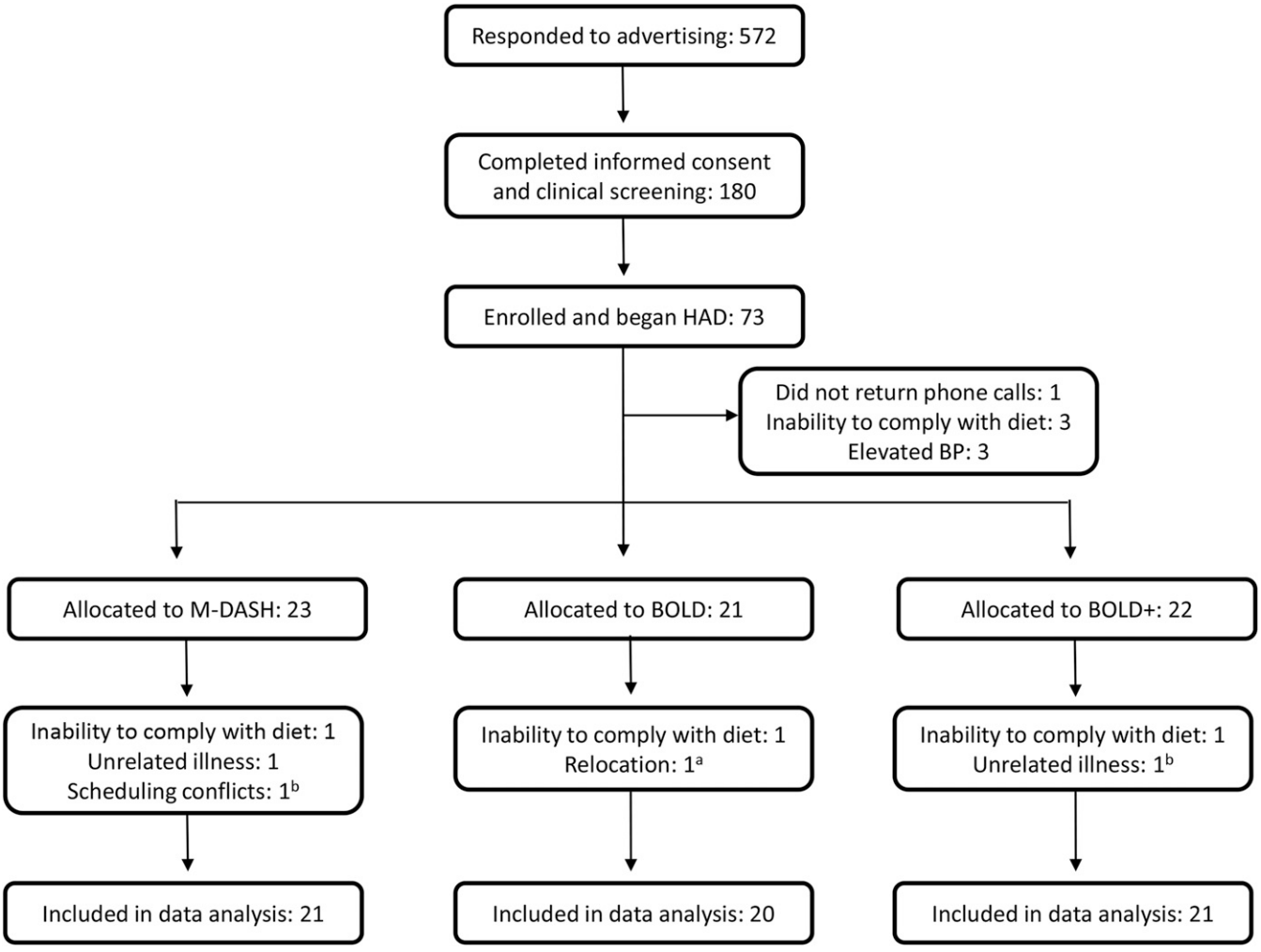

FIGURE 1 Participant flow diagram. [a]Partial data, completed weight-maintenance and weight-loss phases. [b]Partial data, completed weight-maintenance phase. BP, blood pressure; BOLD, Beef in an Optimal Lean Diet; BOLD+, Beef in an Optimal Lean Diet Plus; HAD, healthy American diet; M-DASH, modified Dietary Approaches to Stop Hypertension.

TABLE 4
Screening characteristics of participants in the different diet groups[1]

	M-DASH ($n = 21$)	BOLD ($n = 20$)	BOLD+ ($n = 21$)
Age, y	45.3 ± 6.7[2]	46.2 ± 9.4	46.4 ± 8.5
Sex, M:F, *n*	9:12	9:11	10:11
Caucasian race, *n* (%)	21 (100)	19 (95)	21 (100)
BMI, kg/m^2	34.7 ± 3.6	34.6 ± 3.7	35.1 ± 4.5
Weight, kg	102.1 ± 15.5	101.8 ± 15.6	104.8 ± 17.7
WC, cm	113.5 ± 9.5	113.6 ± 9.2	117.2 ± 10.3
HDL cholesterol, mg/dL	40.4 ± 9.2	42.1 ± 7.9	41.7 ± 10.8
TG, mg/dL	190.7 ± 56.8	182.6 ± 89.3	181.3 ± 75.2
Glucose, mg/dL	104.0 ± 21.6	101.2 ± 18.7	103.1 ± 13.0
SBP, mm Hg	130.0 ± 11.8	125.8 ± 13.3	127.5 ± 12.6
DBP, mm Hg	89.6 ± 6.9	86.9 ± 7.9	85.9 ± 7.5
Taking medications, *n* participants	7	8	7
Blood pressure	4	2	2
Glucose medication	0	1	1
Lipid medication	1	4	2
Depression/anxiety	5	3	4

[1]No differences existed between groups at screening (nonparametric, 2-sided *t* test). BOLD, Beef in an Optimal Lean Diet; BOLD+, Beef in an Optimal Lean Diet Plus Protein; DBP, diastolic blood pressure; M-DASH, modified Dietary Approaches to Stop Hypertension; SBP, systolic blood pressure; TG, triglycerides; WC, waist circumference.
[2]Mean ± SEM (all such values).

being compliant on 70% ± 1%, 77% ± 1%, and 82% ± 1% of study days, respectively. Compliance during the WL phase was 75% ± 1%, 80% ± 1%, and 90% ± 1% for the BOLD, BOLD+, and M-DASH groups, respectively. Weight changes corresponding to the varying energy levels (i.e., WM or WL) during the controlled-feeding diet phases demonstrate an overall high amount of compliance to the study protocol during the controlled-feeding phases (see Weight and body composition, **Figure 2**). Participants did not achieve further weight loss during the FL phase; body weight losses were maintained during this phase.

During the last 2 wk of the WM phase, participants reported walking a mean of 6303 ± 382 steps/d (self-reported pedometer data). During the last week of the WL phase, participants reported walking a mean of 10,536 ± 463 steps/d (self-reported pedometer data), which represented a significant increase in physical activity (WM compared with WL, $P < 0.0001$). Predicted $\dot{V}O_2$ was 18.5 ± 0.6 mL/kg per minute after HAD, which significantly increased to 22.2 ± 0.7 mL/kg per minute after WL (HAD compared with WL, $P < 0.0001$), and the effect was maintained through FL (22.0 ± 0.7 mL/kg per minute; WL compared with FL, P = NS).

Weight and body composition

Weight and body composition mean ± SEM values are presented in **Table 5**. Weight was significantly reduced from HAD by the end of each phase (Figure 2, $P < 0.0001$), with no between-group differences. Significant weight loss (<1.5% of body weight) occurred during the WM phase but was within the acceptable range for a weight maintenance diet (HAD compared with WM, $P < 0.0001$). Loss of ~5% of body weight occurred during the WL phase (WM compared with WL, $P < 0.0001$), which was maintained during the FL phase on all diets (WL compared with FL, P = NS).

Body composition measures were taken after HAD, WL, and FL phases only (Figure 2, Table 4). Body fat decreased by 8–9% in all groups during the WL phase (HAD compared with WL, $P < 0.0001$), and the reductions remained through the FL phase (WL compared with FL, P = NS). Lean body mass decreased by 2–3% during the WL phase (HAD compared with WL, $P < 0.0001$), which was maintained through the FL phase (WL compared with FL, P = NS). Abdominal fat decreased by ~14% (HAD compared with WL, $P < 0.0001$) during WL and abdominal lean mass decreased by 5–7% (HAD compared with WL, $P < 0.0001$); both effects were maintained through the FL phase (WL compared with FL, P = NS).

MetS endpoints

Primary and secondary endpoints are presented in **Table 6**. A significant main effect of phase ($P < 0.01$) was observed for all endpoints except for insulin. Dietary changes implemented during the WM phase did not reduce the number of MetS criteria per participant (HAD compared with WM, P = NS). MetS criteria decreased after the WL phase (HAD compared with WL, $P < 0.01$), and the changes were maintained during the FL phase (HAD compared with FL, $P < 0.01$). The prevalence of MetS was 100% in all groups at screening, but the prevalence in the BOLD group dropped to 70%, BOLD+ to 81%, and M-DASH to 90% after the HAD phase (NS between groups, **Figure 3**). After the WM phase, all groups had a MetS prevalence of 80–90%, which decreased significantly to 50–60% after WL and maintained through FL (χ^2 for phase, $P < 0.0001$). Multivariate logistic regression analysis revealed that weight loss (HAD or WM to WL and HAD or WM to FL) but not diet was significantly associated with resolution of MetS (**Table 7**). Every 1% reduction in body weight (from HAD to WL) was associated with a 39% increase in the odds of having a resolution of MetS, holding the other independent variables constant. A stronger relation was observed for changes in body weight after the WL phase (i.e., WM to WL) and MetS resolution: every 1% reduction in body weight was associated with an 88% increase in the odds of having a resolution of MetS.

WC and triglycerides did not decrease until after the WL phase and thereafter remained stable through the FL (HAD compared with WL, FL, $P < 0.05$). HDL cholesterol decreased during the WM phase (HAD compared with WM, $P < 0.0001$), returned to HAD levels

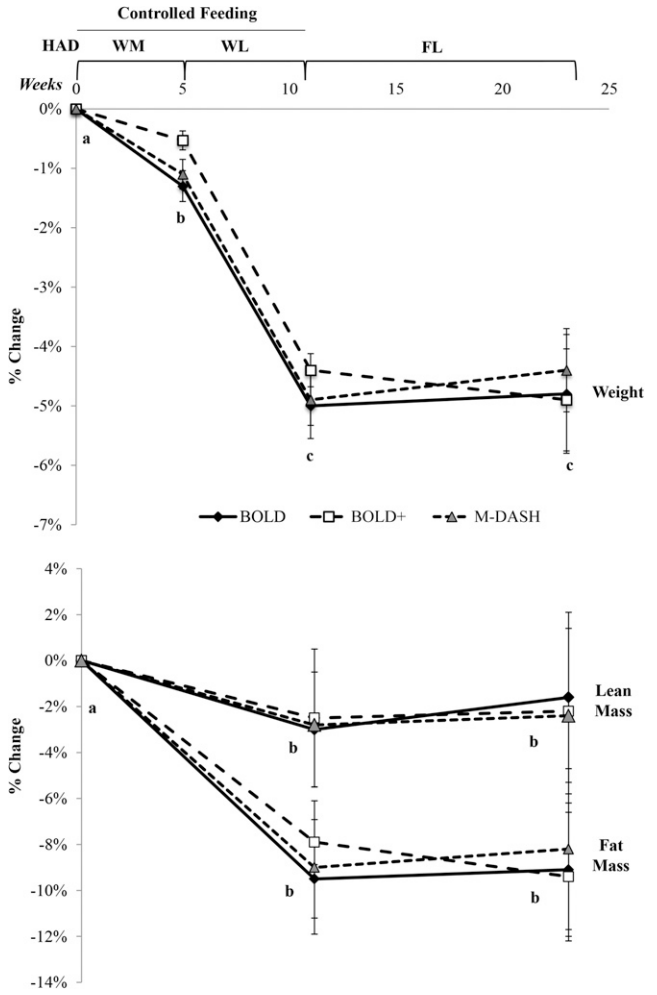

FIGURE 2 Mean ± SEM weight and body composition changes in BOLD ($n = 20$), BOLD+ ($n = 21$), and M-DASH ($n = 21$) diet groups after WM, WL, and FL phases. Different letters denote differences at time points from linear-mixed models adjusted for age and sex; phase, $P < 0.0001$; Tukey-adjusted $P < 0.05$. BOLD, Beef in an Optimal Lean Diet; BOLD+, Beef in an Optimal Lean Diet Plus Protein; FL, free-living weight-loss phase; HAD, healthy American diet; M-DASH, modified Dietary Approaches to Stop Hypertension; WL, weight loss (minimum 500-kcal/d deficit) including exercise phase; WM, weight-maintenance phase.

after the WL phase (HAD compared with WL, P = NS), and increased during the FL phase (WL compared with FL, $P < 0.0001$). Glucose concentrations were not different from HAD after any diet phase, but there was a significant reduction during the WL phase from the WM phase, partially because of a slight increase during WM (WM compared with WL, $P < 0.01$). SBP tended to decrease during the WM phase (HAD compared with WM, $P = 0.07$) and decreased significantly during the WL phase (HAD, WM compared with WL, $P < 0.05$). During the FL phase, SBP increased slightly (although not significantly) from WL (WL compared with FL, P = NS) but remained significantly lower than HAD (HAD compared with FL, $P < 0.01$). DBP decreased only after the WL phase, and the effect was sustained through the FL phase (HAD compared with WL, FL, $P < 0.001$).

Secondary endpoints

TC and LDL cholesterol were reduced after the WM and WL phases (HAD compared with WM, WL, $P < 0.05$) but returned to

TABLE 5
Weight and body composition measurements after each diet phase for M-DASH, BOLD, and BOLD+ diet groups[1]

	M-DASH, mean ± SEM				BOLD, mean ± SEM				BOLD+, mean ± SEM				*P* value		
	HAD	WM	WL	FL	HAD	WM	WL	FL	HAD	WM	WL	FL	Phase × diet	Phase	Diet
Weight, kg	100 ± 2	100 ± 2	96 ± 2	97 ± 2	101 ± 2	100 ± 2	97 ± 2	97 ± 3	105 ± 3	104 ± 3	101 ± 3	100 ± 3	0.89	<0.0001[a]	0.49
BMI, kg/m^2	34.2 ± 0.5	34.1 ± 0.5	32.8 ± 0.6	32.9 ± 0.6	34.4 ± 0.6	34.0 ± 0.6	32.7 ± 0.6	32.8 ± 0.7	35.1 ± 0.7	34.9 ± 0.7	33.6 ± 0.7	33.4 ± 0.8	0.94	<0.0001[a]	0.63
Body fat, kg	35.8 ± 2.0	—	32.5 ± 2.0	32.7 ± 2.1	37.0 ± 1.7	—	33.6 ± 1.8	33.7 ± 2.0	38.2 ± 2.0	—	35.0 ± 2.1	34.2 ± 2.2	0.53	<0.0001[b]	0.50
Body lean mass, kg	59.9 ± 2.4	—	58.8 ± 2.4	58.9 ± 2.5	58.8 ± 2.7	—	57.1 ± 2.8	58.4 ± 2.9	61.0 ± 2.9	—	60.3 ± 3.0	60.4 ± 2.8	0.73	<0.0001[b]	0.57
Percent body fat	35.7 ± 1.6	—	34.2 ± 1.7	34.4 ± 1.8	37.4 ± 1.6	—	35.6 ± 1.8	35.5 ± 1.9	37.1 ± 1.6	—	35.8 ± 1.7	35.3 ± 1.9	0.72	<0.0001[b]	0.12
Abdominal fat, kg	3.9 ± 0.3	—	3.4 ± 0.2	3.5 ± 0.3	4.0 ± 0.2	—	3.4 ± 0.2	3.6 ± 0.2	4.5 ± 0.3	—	3.9 ± 0.2	3.9 ± 0.3	0.88	<0.0001[b]	0.24
Abdominal lean mass, kg	5.9 ± 2.2	—	5.5 ± 0.2	5.4 ± 0.2	5.9 ± 0.3	—	5.5 ± 0.2	5.8 ± 0.3	6.0 ± 0.2	—	5.5 ± 0.3	5.5 ± 0.3	0.73	<0.0001[b]	0.88
Percent abdominal fat	39.6 ± 1.3	—	37.6 ± 1.5	38.1 ± 1.6	39.9 ± 1.1	—	37.8 ± 1.3	37.7 ± 1.4	42.1 ± 1.2	—	40.6 ± 1.3	40.1 ± 1.4	0.81	<0.0001[b]	0.09

[1]Linear mixed model comparing raw values between groups at the end of each diet phase, adjusted for age and sex. Different letters for *P* values denote differences between groups at baseline (HAD). Post hoc Tukey-adjusted $P < 0.05$. [a]HAD compared with WM, WL, FL, Tukey-adjusted $P < 0.05$. [b]HAD compared with WL, FL, Tukey-adjusted $P < 0.05$. BOLD, Beef in an Optimal Lean Diet; BOLD+, Beef in an Optimal Lean Diet Plus Protein; FL, free-living weight-loss phase; HAD, healthy American diet; M-DASH, modified Dietary Approaches to Stop Hypertension; WL, weight loss (minimum 500-kcal/d deficit) including exercise phase; WM, weight-maintenance phase.

TABLE 6
Primary and secondary endpoints after each diet phase for M-DASH, BOLD, and BOLD+ diet groups[1]

	M-DASH ($n = 21$)				BOLD ($n = 20$)				BOLD+ ($n = 21$)				*P* value		
	HAD	WM	WL	FL	HAD	WM	WL	FL	HAD	WM	WL	FL	Phase × diet	Phase	Diet
Primary															
MetS, variables, *n*	3.4 ± 0.2[2]	3.5 ± 0.2	2.9 ± 0.2	2.7 ± 0.2	3.1 ± 0.2	3.3 ± 0.2	2.8 ± 0.2	2.5 ± 0.3	3.1 ± 0.2	3.2 ± 0.2	2.6 ± 0.2	2.9 ± 0.2	0.64	<0.0001[a]	0.58
WC, cm	112 ± 2	112 ± 2	109 ± 2	108 ± 2	113 ± 2	112 ± 2	108 ± 2	106 ± 2	116 ± 3	115 ± 2	111 ± 2	111 ± 2	0.89	<0.0001[a]	0.37
HDL cholesterol, mg/dL	36.3 ± 1.2	33.9 ± 1	36.5 ± 1.3	40.8 ± 1.2	38.4 ± 1.1	35.2 ± 1.1	38.5 ± 1.4	43.3 ± 1.4	40.2 ± 1.7	35.6 ± 1.6	37.1 ± 1.5	41.6 ± 1.5	0.68	<0.0001[b]	0.18
TG, mg/dL	168 (161, 193)[3]	185 (168, 205)	131 (132, 175)	162 (142, 167)	156 (144, 203)	172 (169, 242)	130 (123, 170)	141 (126, 181)	156 (147, 182)	155 (148, 193)	134 (119, 177)	172 (132, 165)	0.82	<0.0001[a]	0.34
Glucose, mg/dL	95 (91, 105)	93 (90, 104)	92 (89, 97)	95 (93, 102)	92 (88, 105)	94 (89, 108)	90 (89, 100)	92 (90, 103)	95 (90, 102)	99 (96, 108)	97 (92, 102)	99 (94, 104)	0.42	<0.0001[c]	0.75
SBP, mm Hg	127 ± 3	124 ± 2	120 ± 2	121 ± 2	122 ± 2	121 ± 2	120 ± 2	123 ± 3	127 ± 2	124 ± 2	120 ± 2	123 ± 3	0.53	<0.0001[d]	0.56
DBP, mm Hg	86.3 ± 1.8	85.6 ± 1.2	82.7 ± 1.4	81.9 ± 1.2	85.3 ± 1.3	83.2 ± 1.6	82.2 ± 1.8	82.1 ± 2.1	85.3 ± 1.6	83 ± 1.5	82.3 ± 1.6	82.6 ± 2	0.84	<0.0001[e]	0.73
Secondary															
TC, mg/dL	198 ± 5	188 ± 5	182 ± 5	203 ± 4	198 ± 8	197 ± 8	183 ± 8	201 ± 7	191 ± 6	177 ± 6	169 ± 7	192 ± 7	0.67	<0.0001[f]	0.27
LDL cholesterol, mg/dL	126 ± 4	117 ± 4	115 ± 4	134 ± 4	126 ± 7	127 ± 8	115 ± 7	127 ± 7	118 ± 5	107 ± 5	103 ± 6	117 ± 5	0.64	<0.0001[f]	0.18
Insulin, mIU/L	7 (5, 9.3)	7 (5.2, 11.9)	4 (4.1, 10.2)	5 (4.6, 9.9)	7 (4.5, 8.9)	5 (4.1, 9.1)	4.5 (3.6, 7)	5 (4.2, 9.4)	8 (6.6, 10.8)	6 (4.6, 9.5)	6 (4.3, 10.6)	5 (4, 9.9)	0.22	0.22	0.63
CRP, mg/L	2.6 (1.8, 5.1)	1.9 (1.6, 4.1)	1.2 (1.1, 4.9)	1.2 (0.9, 4.5)	2.5 (1.9, 4.5)	3.4 (2.5, 5)	2.4 (1.8, 3.9)	3.2 (2.3, 4.3)	2.1 (1.6, 3.7)	2.4 (1.9, 4)	1.7 (1.3, 3.4)	1.7 (1.2, 2.9)	0.18	0.0001[a]	0.4

[1]Linear mixed model comparing raw values between groups from the end of each diet phase, adjusted for age and sex. Post hoc differences for the main effect of phase are represented by the letters. [a]HAD, WM compared with WL, FL, $P < 0.05$. [b]HAD compared with WM, FL; WM compared with WL, FL, $P < 0.05$. [c]WM compared with WL, $P < 0.05$. [d]HAD compared with WL, FL; WM compared with WL, $P < 0.05$. [e]HAD compared with WL, FL, $P < 0.05$. [f]HAD compared with WM, WL; WM, WL compared with FL, $P < 0.05$. BOLD, Beef in an Optimal Lean Diet; BOLD+, Beef in an Optimal Lean Diet Plus Protein; CRP, C-reactive protein; DBP, diastolic blood pressure; FL, free-living weight-loss phase; HAD, healthy American diet; M-DASH, modified Dietary Approaches to Stop Hypertension; MetS, metabolic syndrome; SBP, systolic blood pressure; TC, total cholesterol; TG, triglycerides; WC, waist circumference; WL, weight loss (minimum 500-kcal/d deficit) including exercise phase; WM, weight-maintenance phase.

[2]Mean ± SEM (all such values).

[3]Median; 95% CI in parentheses (all such values). These values are presented because of nonnormally distributed model residuals; log-transformed values are analyzed in model.

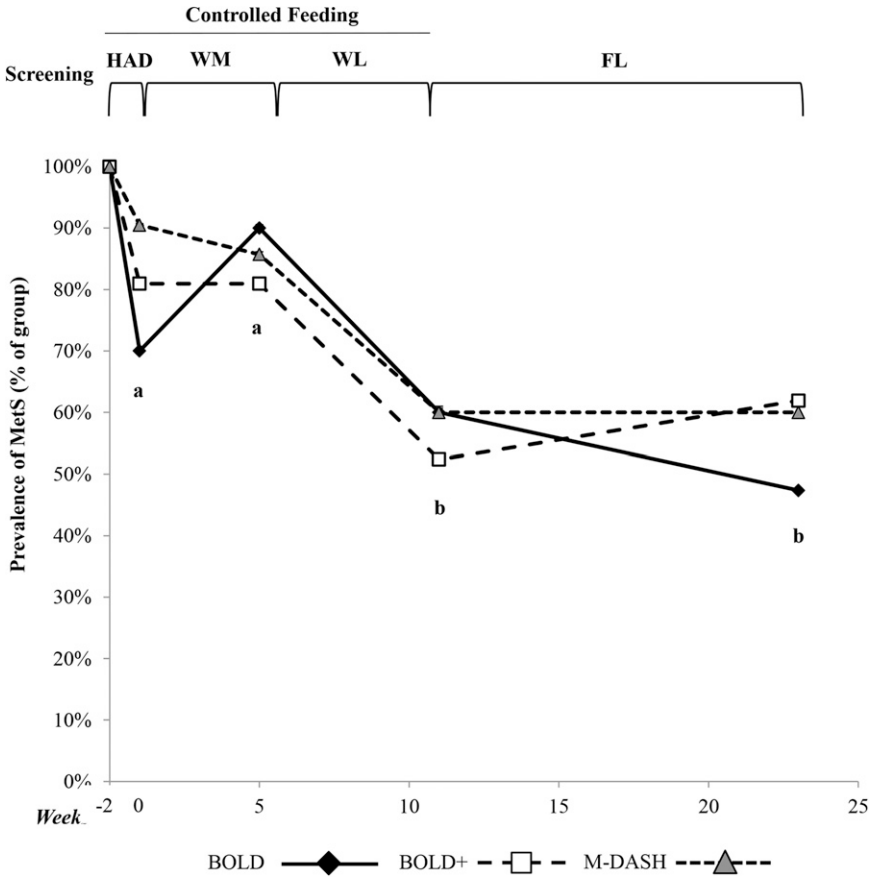

FIGURE 3 MetS prevalence (percentage of group) in BOLD ($n = 20$), BOLD+ ($n = 21$), and M-DASH ($n = 21$) diet groups at screening and after a healthy run-in diet, WM, WL, and FL phases. Different letters denote differences in MetS prevalence by phase, χ^2, $P < 0.0001$. Screening values were not included in the model. BOLD, Beef in an Optimal Lean Diet; BOLD+, Beef in an Optimal Lean Diet Plus Protein; HAD, healthy American diet; M-DASH, modified Dietary Approaches to Stop Hypertension; FL, free-living weight-loss phase; MetS, metabolic syndrome; WL, weight loss (minimum 500-kcal/d deficit) including exercise phase; WM, weight-maintenance phase.

HAD levels after FL (HAD compared with FL, P = NS). C-reactive protein decreased after the WL phase (HAD compared with WL, $P < 0.05$) and remained stable through the FL phase (WL compared with FL, P = NS). Insulin did not change throughout the study (phase, P = NS).

Mean values of EndoPAT measurements are presented in **Table 8**. Augmentation index did not change over time (phase, P = NS), but augmentation index normalized to an HR of 75 beats/min decreased during the controlled-feeding WL phase (HAD compared with WL, $P < 0.01$). A time × treat interaction trend for RHI ($P = 0.09$) and a significant effect for Framingham RHI ($P = 0.04$) were observed; however, post hoc analysis did not reveal where the differences occurred. Framingham RHI decreased nominally on the BOLD diet during the WM and WL phases and increased nominally on the BOLD+ diet during the WM phase, which may account for the significant interaction. Supine SBP and DBP decreased through the WL phase but returned to HAD levels after FL (HAD, FL compared with WL, $P < 0.05$). Baseline HR was significantly lower after the WL phase compared with all other time points ($P < 0.05$).

DISCUSSION

To our knowledge, this is the first study to compare different sources and amounts of protein under controlled-feeding conditions in both weight-stable and weight-loss phases in individuals with MetS. Weight loss was the primary driver of MetS resolution in our study population regardless of protein source or amount. Weight, fat, and lean body mass changes did not differ between diets, with reductions in weight primarily attributed to a loss of body fat. The lack of additional changes in body weight or MetS criteria in the FL phase suggests that participants in this study found it difficult to adhere to the weight-loss recommendations irrespective of the dietary profile. Second, the moderate-protein diet did not confer any advantage (or disadvantage) in improving MetS criteria in either the controlled or FL settings compared with the standard protein diets. Total and LDL cholesterol concentrations responded to the reduction in SFAs in all energy states, whereas HDL cholesterol decreased during WM and increased during the WL and FL phases, possibly as a result of increased physical activity (25).

Diets higher in protein (>25% of calories) are thought to enhance weight loss via a variety of mechanisms: enhanced satiety (26, 27), maintenance of lean body mass and metabolic rate during weight loss (28, 29), and the higher thermic effect of food for protein (30, 31). A meta-analysis of short-term weight-loss studies (32) compared the effects of moderate-protein (25–30% of total calories) with standard-protein diets (12–18% of total calories) matched for energy intake on weight, body composition, metabolic rate, and MetS criteria (32). Beneficial effects (weighted

TABLE 7
Logistic regression model determining factors associated with resolution of MetS[1]

Independent variable	Coefficient (95% CI)	*P* value
HAD to WL		
Weight, % change	0.328 (0.039, 0.618)	0.026
Diet	0.428 (−0.303, 1.158)	0.251
Age	−0.019 (−0.099, 0.061)	0.640
Sex	−1.727 (−3.328, −0.126)	0.034
HAD to FL		
Weight, % change	0.290 (0.093, 0.488)	0.004
Diet	0.370 (−0.417, 1.158)	0.356
Age	−0.038 (−0.130, 0.055)	0.424
Sex	−2.058 (−3.682, −0.434)	0.013
WM to WL		
Weight, % change	0.632 (0.228, 1.036)	0.002
Diet	0.377 (−0.407, 1.162)	0.346
Age	−0.034 (−0.124, 0.056)	0.456
Sex	−2.59 (−4.5, −0.686)	0.008
WM to FL		
Weight, % change	0.361 (0.135, 0.587)	0.002
Diet	0.309 (−0.516, 1.134)	0.463
Age	−0.038 (−0.136, 0.060)	0.450
Sex	−2.359 (−4.092, −0.626)	0.008

[1]Multivariate logistic regression analysis was used to determine associations between independent variables and resolution of MetS from end of HAD or WM to WL or FL. The dependent variable in this analysis is MetS coded so that 0 = has MetS and 1 = does not have MetS. FL, free-living weight-loss phase; HAD, healthy American diet; MetS, metabolic syndrome; WL, weight loss (minimum 500-kcal/d deficit) including exercise phase; WM, weight-maintenance phase.

mean difference) on body weight (−0.79 kg; 95% CI: −1.5, −0.08 kg), fat mass (−0.84 kg; 95% CI: −1.26, −0.48 kg), fat-free mass (0.43 kg; 95% CI: 0.09, 0.78 kg) and triglycerides [−0.23 mmol/L (−20 mg/dL); 95% CI: −0.33, −0.12 mmol/L] were observed for the moderate-protein compared with a standard-protein diet; however, the effect sizes were small, and the analysis excluded studies with a prescribed exercise component. We did not observe any statistical differences between the moderate- and standard-protein groups in any of these endpoints during WL or FL phases, which may be because of the following reasons. First, the controlled-feeding design of this study required participants to consume all foods provided; therefore, a mechanism by which protein enhances weight loss (i.e., the reduced intake of food as a result of enhanced satiation) could not affect outcomes. Second, the high quality of the carbohydrates (rich in fiber and plant protein) in our standard-protein diets may mask the triglyceride decrease that is usually attributed to a reduction in refined carbohydrates (33).

Epidemiologic evidence regarding the impact of red meat intake and CVD remains mixed, with some studies showing an adverse association and others showing none, especially when processed and unprocessed meat are separately categorized (34–39). Micha et al. (40) cited that differences in sodium between processed and unprocessed meat may explain most of the observed higher risk. Several studies have found a link between red meat and MetS (41–43); however, the associations are not always consistent (42, 44), and Damião et al. (45) found that adjusting for SFAs eliminated the association between red meat and MetS. Importantly, the red meat in this study was unprocessed, and lean beef was prepared with

TABLE 8
EndoPAT measurements after each diet phase for M-DASH, BOLD, and BOLD+ diet groups[1]

	M-DASH ($n = 21$)				BOLD ($n = 20$)				BOLD+ ($n = 21$)				*P* value		
	HAD	WM	WL	FL	HAD	WM	WL	FL	HAD	WM	WL	FL	Phase × diet	Phase	Diet
AI	8.7 ± 3.8[2]	5.5 ± 3.1	9.8 ± 4.5	8.3 ± 4.5	7.7 ± 4.9	6.2 ± 3.9	3.9 ± 4.0	4.4 ± 4.0	3.2 ± 3.6	2.1 ± 2.5	2.8 ± 3.2	3.2 ± 3.0	0.35	0.50	0.28
AI@75	3.0 ± 4.2	0.7 ± 3.5	0.6 ± 4.7	2.1 ± 4.3	3.1 ± 4.5	0.3 ± 3.9	−2.5 ± 4.3	−2.2 ± 4.1	−1.0 ± 3.8	−3.4 ± 2.6	−4.6 ± 3.6	−3.6 ± 3.4	0.54	0.02[a]	0.39
RHI	2.0 (1.8, 2.3)[3]	2.0 (1.9, 2.4)	2.1 (1.9, 2.4)	1.9 (1.9, 2.4)	1.9 (1.8, 2.4)	1.8 (1.8, 2.4)	1.8 (1.7, 2.1)	2.2 (1.9, 2.6)	1.9 (1.7, 2.3)	2.2 (2.0, 2.6)	1.9 (1.9, 2.4)	2.1 (1.9, 2.5)	0.09	0.16	0.67
fRHI	0.5 ± 0.1	0.5 ± 0.1	0.6 ± 0.1	0.5 ± 0.1	0.6 ± 0.1	0.4 ± 0.1	0.4 ± 0.1	0.6 ± 0.1	0.4 ± 0.1	0.6 ± 0.1	0.5 ± 0.1	0.5 ± 0.1	0.04[b]	0.79	0.88
SBP, supine, mm Hg	130 ± 3	125 ± 3	124 ± 4	126 ± 3	125 ± 3	124 ± 3	120 ± 3	123 ± 3	130 ± 3	126 ± 3	123 ± 4	130 ± 3	0.71	0.0006[c]	0.50
DBP, supine, mm Hg	74 ± 2	73 ± 2	71 ± 2	73 ± 2	73 ± 2	71 ± 2	69 ± 2	73 ± 3	76 ± 2	73 ± 2	72 ± 2	76 ± 2	0.99	0.0035[c]	0.65
Resting HR, bpm	66 ± 2	64 ± 1	60 ± 2	65 ± 2	67 ± 2	65 ± 11	65 ± 3	64 ± 2	66 ± 2	66 ± 2	63 ± 2	64 ± 2	0.22	<0.0001[d]	0.58

[1]Linear mixed models were used to compare raw values from the end of each diet phase between groups, adjusted for age and sex. [a]HAD compared with WL, $P < 0.01$. [b]No significant Tukey-adjusted post hoc differences. [c]HAD, FL compared with WL, $P < 0.05$. [d]HAD, WM, FL compared with WL, $P < 0.05$. AI, augmentation index; AI@75, augmentation index at heart rate of 75 beats/min; BOLD, Beef in an Optimal Lean Diet; BOLD+, Beef in an Optimal Lean Diet Plus Protein; DBP, diastolic blood pressure; FL, free-living weight-loss phase; fRHI, Framingham reactive hyperemia index; HAD, healthy American diet; HR, heart rate; M-DASH, modified Dietary Approaches to Stop Hypertension; RHI, reactive hyperemia index; SBP, systolic blood pressure; WL, weight loss (minimum 500-kcal/d deficit) including exercise phase; WM, weight-maintenance phase.
[2]Mean ± SEM (all such values).
[3]Median; 95% CI in parentheses (all such values). These values are presented because of nonnormally distributed model residuals; log-transformed values are analyzed in model.

methods that did not include charring. Red meat is generally restricted in a heart-healthy diet because it is a source of SFAs, yet hamburgers and beef dishes contribute fewer SFAs to the US diet than full-fat cheese, pizza, and grain-based desserts (46). Collectively, the evidence to date indicates that the protein source (plant compared with animal or red meat compared with other animal proteins) is secondary to reduced energy and SFA intake for treatment of MetS or CVD risk factors.

We have shown previously that the inclusion of lean beef in a reduced SFA diet lowers TC, LDL cholesterol, and SBP in hypercholesterolemic individuals (12, 13). In the present study, however, we did not observe this in the BOLD diet group. We believe the primary reason was the relatively lower compliance of the BOLD group during WM. Moreover, the participants in the current study did not have elevated LDL cholesterol and were obese. The cholesterol reduction in response to lowering SFAs in the absence of weight loss in obese individuals is blunted compared with normal-weight individuals (47); in addition, lower baseline LDL cholesterol concentrations are correlated with a smaller reduction to a dietary intervention (47). Nonetheless, given the reduction in the BOLD+ group, we believe that the lack of compliance in the BOLD group is the most likely explanation. Nonsignificant changes in triglycerides also were observed during WM; triglycerides remained stable in the BOLD+ group but increased in the BOLD and M-DASH groups. This is consistent with the OmniHeart Trial, which showed that replacement of dietary carbohydrate with protein lowers triglycerides (6). Any differences observed between groups during the WM phase were nullified during WL, which significantly reduced cholesterol and triglyceride concentrations in all groups.

A limitation of this study is that it was not originally powered to detect differences between groups but rather differences between the HAD and the experimental diets (considered within-group changes). Power calculations were based on BP and triglyceride changes in hypertensive or hypercholesterolemic individuals rather than individuals who were obese or had MetS because of a lack of appropriate comparative studies at the time of study design. Although recruitment goals were not met, there were significant improvements for most risk factors and no strong trends in between-group comparisons of primary endpoints that presumably may have reached significance with additional participants. We observed only small changes in SBP during weight maintenance under the current experimental conditions: −3.05 mm Hg for M-DASH, −3.19 mm Hg for BOLD+, and −1.65 mm Hg for BOLD. The magnitude of these changes was similar to those reported by Appel et al. (7) in normotensive individuals following the DASH diet (SBP: −3.5 mm Hg; DBP: −2.1 mm Hg). We estimate that sample sizes in excess of 55,976 and 557 persons per group would be required for the observed between-group differences of 0.14 mm Hg and 1.4 mm Hg for SBP (BOLD+ compared with M-DASH and BOLD compared with M-DASH, respectively, during WM) to have been statistically significant (80%, $P < 0.05$). Furthermore, the effect size for these differences was very small (Cohen's d was 0.02 and −0.17, respectively) (48). This suggests that a larger controlled-feeding study evaluating differences in dietary protein sources is not warranted given the small clinical differences. More substantial reductions were observed after WL (−6.9 mm Hg in M-DASH, −7.4 mm Hg in BOLD+, and −2.4 mm Hg in BOLD). These findings support the current recommendations for weight loss for the treatment of MetS in overweight/obese individuals (4), indicating that even small changes in body weight (−5%) elicit clinically meaningful improvements in metabolic outcomes. Although well matched for macronutrient composition, the M-DASH diet was considerably higher in dietary fiber, yet all diets exceeded current intake recommendations (46). Increasing dietary fiber is associated with small reductions in BP (49, 50), TC, and LDL cholesterol (51) and improved glycemic control in patients with type 2 diabetes (52, 53) but does not affect triglycerides and HDL cholesterol (51). Because we did not observe significant differences between the BOLD and M-DASH diets in key biomarkers shown to be responsive to increases in dietary fiber, it is unlikely that the increased dietary fiber beyond recommended intakes in M-DASH affected MetS outcomes. There is little research examining the effects of dietary fiber intakes far beyond recommendations. It is possible that there is a threshold effect for dietary fiber with limited effects on MetS criteria beyond that observed with current recommendations for a dietary pattern rich in fruits, vegetables, and whole grains (54). Our study coordinator and data analyst were not blinded, but the statistics were performed by an independent coauthor by using multiple models, and the same conclusions were made. Because dietary data were unavailable during the FL phase, we were limited to changes in weight and MetS criteria for evaluating adherence. Finally, a biomarker of protein intake was not measured to assess compliance; however, a controlled-feeding design lessens the necessity for this.

This study adds to the literature in that it confirms that weight loss via diet and exercise is the primary treatment for MetS. By using a tightly controlled study design, the probability of noncompliance and confounding variables affecting these results is much lower than in studies with free-living participants. A run-in phase also reduced the variability of the baseline data and culled noncompliant individuals. The diets were matched for dietary factors that affect cholesterol, such as SFA and soy, to isolate the effects of plant compared with animal protein. We used highly sensitive methods for endpoint testing to reduce the variability in the data, such as DXA scans for body composition measurements. Moreover, the exercise component was measured objectively with pedometers and predicted $\dot{V}O_2$. This is one of the first studies designed to compare 2 diets rich in fruits and vegetables and low in SFAs but matched in protein from different sources (i.e., plant and animal proteins). In conclusion, this study is clinically relevant because it demonstrates that weight-loss diets low in SFAs that incorporate either plant or animal proteins (i.e., lean beef) are effective for treating MetS criteria without adverse effects on other important risk factors for CVD (i.e., LDL cholesterol and TC concentrations).

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